Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden

Effect of xylitol varnishes on remineralization of artificial enamel caries lesions *in vitro*

C.A.B. Cardoso [1], A.R.F. de Castilho [1], P.M.A. Salomão, E.N. Costa, A.C. Magalhães, M.A.R. Buzalaf [*]

Department of Biological Sciences, Bauru Dental School, University of São Paulo, Al. Octávio Pinheiro Brisolla, 9–75, Bauru 17012-901, SP, Brazil

ARTICLE INFO

Article history:
Received 6 May 2014
Received in revised form
5 August 2014
Accepted 15 August 2014

Keywords:
Dental caries
Dental remineralization
Fluoride varnish
Xylitol

ABSTRACT

Objectives: Analyse the effect of varnishes containing xylitol alone or combined with fluoride on the remineralization of artificial enamel caries lesions *in vitro*.

Methods: Bovine enamel specimens were randomly allocated to 7 groups ($n = 15$/group). Artificial caries lesions were produced by immersion in 30 mL of lactic acid buffer containing 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM KH_2PO_4, 6 μM tetraetil metil diphosphanate (pH 5.0) for 6 days. The enamel blocks were treated with the following varnishes: 10% xylitol; 20% xylitol; 10% xylitol plus F (5% NaF); 20% xylitol plus F (5% NaF); Duofluorid™ (6% NaF, 2.71% F + 6% CaF_2), Duraphat™ (5% NaF, positive control) and placebo (no-F/xylitol, negative control). The varnishes were applied in a thin layer and removed after 6 h. The blocks were subjected to pH-cycles (demineralization—2 h/remineralization—22 h during 8 days) and enamel alterations were quantified by surface hardness and transversal microradiography. The percentage of surface hardness recovery (%SHR), the integrated mineral loss and lesion depth were statistically analysed by ANOVA/Tukey's test or Kruskal–Wallis/Dunn's test ($p < 0.05$).

Results: Enamel surface remineralization was significantly increased by Duraphat™, 10% xylitol plus F and 20% xylitol plus F formulations, while significant subsurface mineral remineralization could be seen only for enamel treated with Duraphat™, Duofluorid™ and 20% xylitol formulations.

Conclusions: 20% xylitol varnishes seem to be promising alternatives to increase remineralization of artificial caries lesions. Clinical significance: effective vehicles are desirable for caries control. Xylitol varnishes seem to be promising alternatives to increase enamel remineralization *in vitro*, which should be confirmed by *in situ* and clinical studies.

1. Introduction

The use of xylitol has shown to have an important influence on the control of risk factors and prevention of dental caries.[1–3] Clinical results of studies held in Turku showed a 85% decrease in the incidence of dental caries when using chewing gum containing xylitol compared to those with sucrose[4–6] and a caries decrease between 30 and 60% in a study held with Finnish children.[7] However, there is uncertainty about the real mechanism of action of xylitol involved in caries control.

* Corresponding author. Tel.: +55 14 32358346; fax: +55 14 32271486.
E-mail addresses: mbuzalaf@fob.usp.br, mabuzalaf@yahoo.com (M.A.R. Buzalaf).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.jdent.2014.08.009
0300-5712/

Many studies report a reduction in salivary levels of *Streptococcus mutans* due to the prolonged and continuous exposure to xylitol by chewing gums, indicating that this polyol can decrease the ability of the bacteria to multiply.[8–10] However, the clinical relevance of reduced intra-oral levels of this microorganism is still unclear[11] as not all studies confirm the inhibitory effect of xylitol on *S. mutans*.[12]

Other probable mechanism of action of xylitol is on enamel remineralization. It has been shown to have the ability to form complexes with calcium ions on the dental surface, inhibiting the translocation of dissolved calcium and phosphate, and the resultant demineralization.[13,14] An study involving high-resolution electron microscopy and microradiography revealed a higher remineralization in intermediate and deep layers of enamel samples immersed in 20% xylitol solution compared with control.[15]

High frequencies of intake of xylitol have been employed in most of the studies,[3,7,9] due to the rapid clearance of xylitol from the oral cavity. However, these protocols could rely on patients' compliance, taking into consideration the price and number of times that these vehicles should be used daily to maintain salivary levels of xylitol able to control dental caries. The efficacious dose range for xylitol consumption has been determined to be 6–10 g per day, based on data showing suppression of salivary *S. mutans* counts,[16] which would require consumption of at least five 2-g pieces of gum with xylitol as the sole polyol/day to reach the daily dose.[17] Therefore, alternative vehicles are being evaluated as well as the optimal dose required for caries control. *In vitro* and *in situ* studies with xylitol solutions containing concentrations of this polyol ranging from 0.5% to 65%, as well as clinical studies using tablets, artificial saliva, childrens' syrup, toothpaste and chewing gum containing varying doses of xylitol have been reported.[7,18–23]

Dental varnish is a widely used strategy of topical fluoride application to control caries, as its adherence to tooth surface leads to longer maintenance of fluoride levels in the oral cavity. The most effective varnish reported in the literature has sodium fluoride as anticariogenic agent.[24–26] Xylitol-containing varnishes have been recently developed. They were shown to be effective to increase salivary xylitol levels[27] and to reduce erosion.[21] However, their effect in the remineralization of caries lesions has never been evaluated so far. Thus, the present *in vitro* study aimed to analyse the remineralizing effect of experimental varnishes containing xylitol alone or combined with fluoride on artificial enamel caries lesions *in vitro*, considering its mechanism of action on tooth minerals and not on bacteria. The null hypothesis was the varnishes containing xylitol (with or without fluoride) are as effective as commercial fluoride varnishes on enamel remineralization.

2. Materials and methods

2.1. Preparation of bovine enamel specimens and artificial caries formation

One hundred and thirty enamel specimens (4 mm × 4 mm × 2.5 mm) were prepared from incisor bovine teeth, freshly extracted, disinfected by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. After visual inspection, stained and/or cracked teeth were excluded. Besides, soft tissues were removed from the coronal and root surfaces with the aid of a periodontal curette (Duflex™, SSWhite, Rio de Janeiro, RJ, Brazil). The specimen was obtained, after two double sections of the widest portion of the dental crowns, and polished, as described by Magalhães et al.[28]

One hundred and five enamel specimens were selected by using the baseline surface hardness (mean KHN 346 ± 27), they had 1/3 of the surface protected (control area) with nail varnish and they were further subjected to the formation of artificial caries lesion by immersion in 30 mL of buffer containing 50 mM lactic acid, 3 mM $CaCl_2{\cdot}2H_2O$, 3 mM KH_2PO_4, 6 μM tetraetil metil diphosphanate and traces of thymol (KOH to adjust pH to 5.0)[29] for 6 days. After demineralization, the other outer 1/3 of the surface was protected with nail varnish (demin control area).

2.2. Treatment and pH-cycling

Five experimental varnishes (control, containing 10% and 20% xylitol with or without fluoride), with the same basic composition as the commercial varnish, were especially manufactured by FGM/Dentscare (Joinville, SC, Brazil). Xylitol concentrations were determined by the maximum incorporation of that polyol into the varnish that would not lead to precipitation. The varnishes contained colophonium, synthetic resin, thickening polymer, essence and ethanol (informed by manufacturer). Xylitol was supplied by Danisco (Xylitab™ 300, Danisco Brasil Ltda, Cotia, SP, Brazil).

Enamel specimens with mean %SHC of 81.2 ± 9.5 were selected and randomly allocated to 7 different groups (n = 15/group), according to the type of varnish that would be applied: (1) Duraphat™ (5% NaF, 2.26%F, pH 5.0, Colgate, São Bernardo, SP, Brazil); (2) Duofluorid™ (6% NaF, 2.71% F, 6% CaF_2, pH 8.0, FGM/Dentscare); (3) 10% xylitol (pH 5.0, FGM/Dentscare); (4) 20% xylitol (pH 5.0, FGM/Dentscare) (5) 10% xylitol + 5% NaF (pH 5.0, FGM/Dentscare); (6) 20% xylitol + 5% NaF (pH 5.0, FGM/Dentscare); (7) no xylitol or fluoride (pH 5.0, control; FGM/Dentscare). A thin layer of varnish was applied using microbrush on the demineralized enamel samples and they were immediately immersed in artificial saliva (0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2{\cdot}2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and traces of ascorbic acid, pH 6.8; 30 mL per sample)[30] at 25 °C for 6 h.[28] The varnishes were then carefully removed using a surgical blade and cotton swabs soaked in 50% acetone solution.[31]

The blocks were then subjected to a pH-cycling model, during 8 days, according to Queiroz et al.[32] During 8 days, the blocks were kept for 2 h in the demineralizing solution (0.05 mol/L acetate buffer, pH 5.0 and containing 1.28 mmol/L Ca, 0.74 mmol/L P and 0.03 μg F/mL) and for 22 h in remineralizing solution (1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, 0.05 μg F/mL in 0.1 mol/L Tris buffer, pH 7.0) at 37 °C. The proportions of demineralizing and remineralizing solutions per area of enamel were 6.25 mL/mm^2 and 3.12 mL/mm^2, respectively. On the fourth day, the de- and